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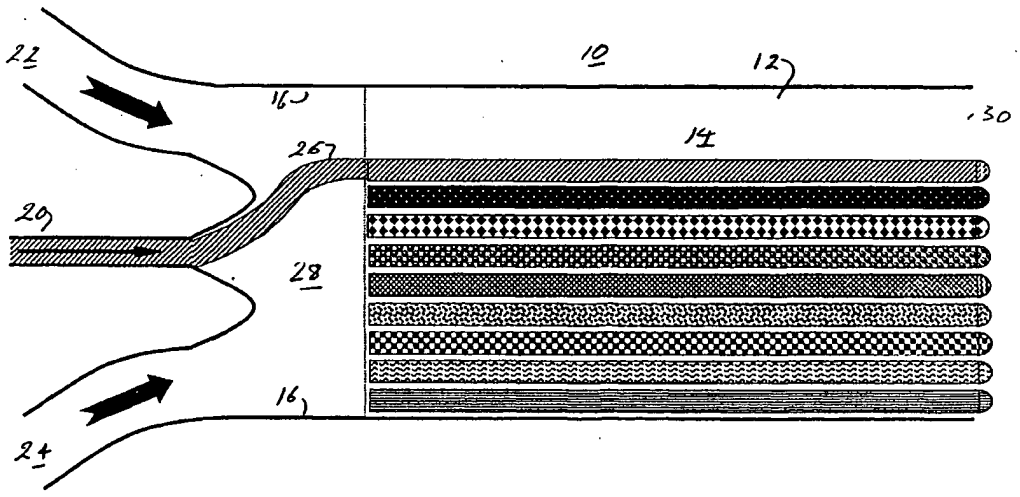

(57) Abstract

A composition of a liquid (26) is caused to interact with a narrow band shaped area at a chosen position on a solid surface within a flow channel (12) by hydrodynamic focusing of a guided stream of said liquid between two streams of guiding liquid (28). By altering the ratio of the flow rates of the two guiding liquid streams, the position of the guided liquid stream is changed and further interaction with the solid surface takes place along a second band shaped area. Using two such flow channels it is possible to produce a two dimensional array of interaction sites.

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Spatially directed interaction on a solid surface

The present invention relates to methods for performing interactions between a liquid or a component of the liquid and a solid surface, such as chemical reactions which may for instance be immobilisation or synthesis reactions on a solid surface, in which the location at which the interaction occurs is spatially defined by hydrodynamic focusing or by electrodynamic focusing.

There is a requirement for being able to position or synthesise different chemical species in an array of row or spot locations on a micro-scale on the surface of a solid substrate. For instance, in conducting DNA hybridisation studies there are proposals which require a "chip" having a surface on which every possible combination of a number of DNA bases is represented at a known and defined location, for instance every possible 8-mer.

Various screen printing techniques have been proposed in the past for making such arrays. For instance US 5412087 is one example out of very many that describe methods of building such arrays by protecting a layer of chemical monomer sub-units put on a surface with a photo-labile protecting group, using a photo-mask to remove the protection at selected locations by light exposure and reacting the next layer of chemical sub-units with the last layer at the unprotected locations. This process is repeated over and over to produce an array having a polymer synthesised on the surface and composed of different sequences of monomer units (typically amino acids or nucleotide bases) at known row-column locations of the array.

It is known from "Biosensors and Bioelectronics Vol.13 No. 3-4, pages 427-438, 1998" that one can on a micro-scale choose the lateral position within a flow path occupied by the flow of a particular stream of liquid by altering the proportion of a guide liquid which is introduced on either side of the stream of interest. This technique is termed 'hydrodynamic focussing'. The term 'hydrodynamic' is of course not to be understood as implying that the liquids employed must necessarily be wholly or in part aqueous. Thus, it is disclosed there in relation to a flow switch that a flow of switchable liquid flowing in a covered trench 400 μm wide and 50 μm deep on the top of a silicon substrate can be guided to flow nearer either edge of the trench as desired. The flow is introduced through an inlet aligned with the centre line of the trench. Two other inlets on either side of first inlet introduce a guide buffer flow. By choosing what proportion of the guide buffer flow comes in on each side without altering the total amount of guide buffer flow, the lateral position of the switchable flow within the trench is varied. This determines through which of two outlets at the other end of the trench the hydrodynamically focused switchable flow exits.

Due to the small scale of the device, the Reynolds number of the flow is low and pure laminar flow is obtained. There is therefore no mixing other than by diffusion between the guide buffer flows and the switchable flow.

WO98/10267 (Technical University of Denmark) describes passing a fluid containing particles through a micro flow channel guided between two buffer streams. The position of the particle containing flow stream within the channel may be

adjusted by regulation of the volumetric ratio between the flow rate of the particle containing flow and the guiding buffer flows. The cross-section of the particle containing flow may thereby be regulated to be little more than the diameter of the particles, so that they pass one at a time.

Particles may be dragged out of the particle containing flow by laterally disposed magnets. Alternatively electrodes may be used to separate particles by electrophoretic or dielectrophoretic forces. Particles may be temporarily held in fixed positions in the flow channel by electrodes to allow chemical reactions to take place.

Alternatively, a field may be generated along the flow channel, as by a magnet at one end of the channel, and particles may be caused to migrate along the channel according to their susceptibility to the field.

Particles may be deflected to an outlet which is opened only in response to the presence of such a particle. Particles may be entrapped by a magnet and then released to flow to an outlet at a high concentration.

Different sub-populations of particles may be sorted to emerge via multiple outlets.

It is further disclosed that a microchip bearing an array of oligonucleotides can be placed in the micro flow channel system allowing several steps of washing, staining and reagent addition to be integrated in an automated routine in a flow through mode. Immunosensor arrays can be used in the same way. It is further disclosed that the channel may have a plurality of assay sites located in the channel and comprising immobilised reagents. Field generating means may be provided at the assay sites for attracting and

immobilising reagents or rejecting them from the site. Such sites may form a two-dimensional grid of sites bearing respective reagents.

The references in WO98/10267 to the position of the particle containing flow within the channel being adjustable by regulation of the buffer flows are not explained further but appear to relate to those embodiments in which the outlet flow of the streams is switched between first and second outlets to operate as a cell sorter.

The two-dimensional array described with reference to Figure 14 is apparently to be within the central flow so that all of the array sites are simultaneously addressed by the flow. For addressing separate streams to different array sites, an arrangement having multiple parallel flow channels is described with reference to Figure 16.

Kenis et al (Paul A. Kenis, Rusten F. Ismagilov, George M. Whitesides: Science Vol. 285, 2 July 1999) published after the priority dates hereof, described the use of a guided, focused stream to carry out various reactions along a path over a solid surface, with positioning of the fabricated features to within a few percent of the channel width.

Electrodynamic focusing (also known as electrokinetic focusing) is described in US-A-5858187. It is disclosed that a flow of sample material across an intersection formed by a sample channel and two focusing channels can be confined to less than the width of the sample channel and that the focused flow can be laterally guided. Two modes of operation are disclosed. In one mode, there is a flow of sample liquid which is focused by two streams of focusing liquid produced by electro-osmotic forces. In an alternative mode, the

surface of the channel is coated to block the production of electro-osmotic force and liquid flow is prevented or is minimised. However, ionically charged materials in the sample are transported by electrophoretic force and follow lines of electric potential which produce focusing of the sample material.

It is desirable to have available a wider range of 'wet chemistry' reaction techniques for immobilising reagents or reactants or carrying out chemical synthesis than are consistent with the use of photo-labile protecting agents described above. Generally, conventional "wet chemistry" synthesis techniques offer inherently better yields than photo-chemical methods. Furthermore, such "wet chemistry" methods are better suited to providing in-process quality control by measuring the reactants present on an on-going basis. Furthermore, there is a much more substantial knowledge base available, allowing better optimisation for specific uses and conditions. It is also desirable to have a means of carrying out a wider range of site directed solid-liquid interactions such as magnetic or electrostatic capture interactions than is offered by the prior art.

The present invention now provides according to a first aspect a method for producing an interaction between a hydrodynamically focused liquid or a component of said hydrodynamically focused liquid and a selected region of a target surface comprising:

providing said target surface as part of one of a plurality of surfaces together defining a flow path for liquid, the surface containing the target surface serving to define the width of the flow path,

providing for said flow path a set of at least three fluid inlets and at least one fluid outlet such that a flow of said hydrodynamically focused liquid can be directed into the flow path through one said inlet guided between two flows of guidance liquid introduced via the two other said inlets to leave said flow path through said at least one outlet,

providing for said two guidance liquid inlets flow control means such that the proportion of the total flow of guidance liquid introduced on each side of the said hydrodynamically focused liquid can be varied to position the flow of said hydrodynamically focused liquid laterally within the flow path, and

directing a flow of said hydrodynamically focused liquid and two flows of guidance liquid through respective ones of said inlets and along said flow path such that the flow of said liquid is directed over a selected region of said target surface having a width less than the width of the target surface and extending at a selected lateral position within said flow path controlled by selection of an appropriate flow ratio of guidance liquid introduced on either side of the flow of said hydrodynamically focused liquid,

and allowing said hydrodynamically focused liquid or a component thereof to interact with said selected region of said target surface.

Preferably, the method further comprises re-adjusting the flows of guidance liquid such that the flow of said liquid is directed over a second selected region of said target surface and allowing said hydrodynamically found liquid or a component thereof to interact with said second selected region of said surface. Preferably, said process of re-adjustment and interaction with a further selected region of the surface is repeated multiple times, e.g. at least 3 times, preferably at least 5 times, using the same target surface.

Said interaction between said hydrodynamically focused liquid and said target surface may involve a chemical reaction. However, it should be appreciated that a wide range of forms of interaction are included. Thus, the interaction may be the capture of a species in the liquid by a magnetic field attracting said species to the surface. It may be the capture of a species in the liquid to the surface by virtue of an inherent affinity, whether chemical or physical, between the surface and the substance. The term 'surface' in this context is not restricted to the upper boundary of the bulk substrate defining the flow channel but includes any material already present thereon. The affinity may be due to the presence of one or more sources of magnetic attraction associated with the surface. Thus, one or more magnets may be used to attract paramagnetic beads out of a suspension in a liquid directed over a target region of the magnetically attractive surface. Similarly an electrically charged surface may be used to attract and hold electrostatically or dielectrically attractable particles out of a liquid. Generally however, it will be understood that

when the interaction in question is in the nature of a binding of the substance in question to the surface it will be of a desired and generally specific nature and will not include a random or non-specific binding to the surface.

Where a chemical reaction is responsible for the interaction it may involve the formation of covalent or non-covalent bonds and may or may not involve the immobilisation of any substance to the surface as a result of the interaction. Thus, antibody/antigen specific binding interactions or oligonucleotide hybridisation reactions are contemplated as well as covalent synthesis reactions. However, equally contemplated are chemical reactions in which a compound is stripped from a surface, such as the surface site specific dehybridisation of oligonucleotides or disruption of antibody/antigen complexes by chaotropic agents or the cleavage of a bound enzyme substrate by an enzyme in the liquid. Also contemplated are reactions in which a component of the liquid reacts with the surface without binding, such as the reaction of an enzyme substrate in the liquid with an enzyme bound on the surface or vice versa.

It will be appreciated that the techniques described herein are of enormously wide applicability and the above broad examples are merely a few of the types of interaction contemplated.

Where said interaction is a chemical reaction it may therefore be an immunoaffinity reaction, a nucleotide hybridisation reaction, a chemical synthesis reaction, a chemical deprotection reaction, an enzyme catalysed reaction, or an enzyme inhibition reaction amongst other possibilities. Said reaction may comprise immobilising a first nucleotide or

oligonucleotide on said surface. It may comprise covalently adding a further nucleotide or oligonucleotide to a nucleotide or oligonucleotide already immobilised on said surface.

Said reaction may comprise immobilising a first amino acid residue or a peptide on said surface. It may comprise covalently adding a further amino acid or peptide to one already immobilised on said surface.

In order to preserve laminar flow of the guide and hydrodynamically focused flow streams the flow of liquid through said flow path is preferably at a Reynolds number of no more than 10, but often it will be much less than this, for instance no more than 5, and generally no more than 1.

The linear flow rate through the flow channel may be quite high even at these low Reynolds numbers because of the small channel dimensions. Thus by way of example with a channel 200 μm wide and 40 μm deep (cross sectional area 8000 μm^2) the volume flow rate might suitably be from 0.1 to 100 μl/min. which corresponds to a linear flow rate of from 0.01 to 10 m/min.

The flows of hydrodynamically focused liquid and of guidance liquid may be produced by mechanical pumps, which preferably are electrically operated. However, the flow required for hydrodynamic focusing may alternatively be produced by electro-osmotic forces. If desired, the flow of one or more of the liquid streams may be produced by a pump and the flow of one or more of the other liquid streams may be induced by electro-osmotic force.

The substrate in which said flow channel is formed may be made from any one of a large number of materials.

Desirable properties are a high level of rigidity and the ability to present an extremely smooth surface e.g. with better than ± 3 μm, preferably better than ± 1 μm, most preferably no more than ± 0.5 μm roughness. For many applications chemical inertness and an absence of non-specific binding for the hydrodynamically focused liquid or its components will be desired. Generally the substrate should lend itself to the formation therein of a precisely dimensioned trench either by etching or an alternative process of material removal or by moulding. Suitable materials include glass, fused silica or silicon, in which substrates the flow channel may be formed as a trench by etching.

An alternative preferred material is an engineering polymer such as polycarbonate, polypropylene, or polymethyl-methacrylate which can be precisely injection moulded to form a shaped substrate providing such a trench for the flow channel. Such materials may be injection moulded using a nickel casting of an etched silicon master.

The surface of the substrate may be modified to increase its smoothness, to alter its hydrophobicity (e.g. by plasma polymerisation of plastics) or its chemical inertness (e.g. by gold coating by vacuum deposition), if desired in selected locations.

Where electro-osmotic force is used to produce liquid flow, it will be necessary for the surface of the substrate to be electrically charged when in contact with the liquid. For instance, silica is a suitable surface for this purpose as when in contact with an aqueous buffer liquid it will bear charged $Si-O^-$ ions. Generally, materials useful in capillary

electrophoresis will be suitable as substrates for use acccrding to this aspect of the invention.

A roof may then provided over the trench to complete the flow channel, suitably by attaching a cover plate by adhesive. The cover plate may be of the same types of materials as are mentioned above but need not specifically be of the same material as the substrate containing the trench. The cover plate may be supported at a constant height across the width of the trench by pillar members. The underside of the cover may be used as the surface on which the liquid interacts instead of the floor of the trench.

The method may include introducing multiple guided streams, each being contained between a respective pair of guiding streams. Adjacent guided streams may be separated by a single guiding stream and only the outermost guiding streams need have their flow rates selected to position the guided streams laterally in the flow channel. All of the guided streams with the guiding streams which lie between them may be moved laterally together, maintaining a constant spacing between the guided streams.

A further guiding stream may be introduced to flow between a base wall of the flow path and the other guiding streams and the guided stream(s). Also, a still further guiding stream may be introduced to flow between a top wall of the flow path and the other streams. The flow rate of these lower and/or upper guiding streams may be varied to adjust their thickness and may be interrupted to allow the other streams to contact the top or base wall as desired.

Following the production of said interaction at said selected region of said target surface, a second interaction

may be conducted between a product of said first interaction and a second hydrodynamically focused liquid or component thereof at a selected sub-region forming part of said selected region by a method comprising:

providing a second plurality of surfaces together defining a second flow path for liquid flow such that one of said surfaces intersects and has a portion in common with said target surface, the dimension of said one surface transverse to the direction of said second flow path defining the width of the second flow path,

providing for said second flow path a second set of at least three fluid inlets and at least one fluid outlet such that a flow of said second hydrodynamically focused liquid can be directed into the flow path through one said inlet guided between two flows of guidance liquid introduced via two other said inlets to leave said flow path through said at least one outlet,

providing for said two guidance liquid inlets of the second flow path flow control means such that the proportion of the total flow of guidance liquid introduced on each side of the said second hydrodynamically focused liquid can be varied to position the flow of said second hydrodynamically focused liquid laterally within the second flow path, and

directing a flow of said second hydrodynamically focused liquid and two flows of guidance liquid through

respective ones of said inlets and along said second flow path such that the flow of said liquid is directed over a selected sub-region of said common portion of said target surface having a width less than the dimension of the common portion of the target surface in the width direction of the second flow path and lying at a selected lateral position within said second flow path controlled by selection of an appropriate flow ratio of guidance liquid introduced on either side of the flow of said second hydrodynamically focused liquid,

and allowing said second hydrodynamically focused liquid or a component thereof to interact with the product of said first interaction on said selected sub-region of said target surface.

Thus according to a second aspect of the invention there is provided a method for producing an interaction between hydrodynamically focused liquids or components of said hydrodynamically focused liquids at a selected region of a target surface comprising:

providing said target surface at an intersection formed by two crossing flow paths defined by respective sets of flow path bounding surfaces,

each said set of bounding surfaces comprising a surface having a width that defines the width of its respective flow path, the target surface being defined by the intersection of said width defining surfaces,

providing for each flow path a set of at least three fluid inlets and at least one fluid outlet such that for each flow path, a flow of hydrodynamically focused liquid can be directed into the flow path through one said inlet guided between two flows of guidance liquid introduced via two other said inlets to leave said flow path via said at least one outlet,

providing for said two guidance liquid inlets of each flow path control means such that the proportion of the total flow of guidance liquid introduced on each side of the hydrodynamically focused liquid can be varied to position the flow of hydrodynamically focused liquid laterally within the respective flow path,

directing a flow of a first hydrodynamically focused liquid along one of said intersecting flow paths to carry out a first interaction between said first hydrodynamically focused liquid or a component thereof and the target surface along a line extending at a selected lateral position within said flow path controlled by selection of an appropriate flow ratio of guidance liquid introduced on either side of the flow of hydrodynamically focused liquid, said interaction producing a product on said target surface,

stopping flow through said one flow path,

directing a flow of a second hydrodynamically focused liquid along the other one of said intersecting flow

paths to carry out a second interaction between said second hydrodynamically focused liquid or a component thereof and the product of said first interaction on said target surface at a point within the intersection of the flow paths lying along a line extending at a selected lateral position within said other flow path controlled by selection of an appropriate flow ratio of guidance liquid introduced on either side of the flow of said second hydrodynamically focused liquid,

whereby said second interaction takes place at a selected location within the area of intersection of the two flow paths defined by the selected lateral positions of said hydrodynamically focused liquid flows.

The invention includes apparatus for use in accordance with the second aspect of the invention by producing an interaction between hydrodynamically focused liquids or components of said hydrodynamically focused liquids at a selected region of a target surface comprising:

a substrate defining said target surface at an intersection formed by two crossing flow paths defined by respective sets of flow path bounding surfaces of the substrate,

each said set of bounding surfaces comprising a surface having a width that defines the width of its respective flow path, the target surface being defined by the intersection of said width defining surfaces,

a set of at least three fluid inlets and at least one fluid outlet associated with each said flow path such that for each flow path, a flow of hydrodynamically focused liquid can be directed into the flow path through one said inlet guided between two flows of guidance liquid introduced via two other said inlets to leave said flow path via said at least one outlet, and

flow control means associated with said two guidance liquid inlets of each flow path such that the proportion of the total flow of guidance liquid introduced on each side of the respective hydrodynamically focused liquid can be varied to position the flow of hydrodynamically focused liquid laterally within the respective flow path.

Multiple inlets for guided flows with intervening guidance flows may be provided as previously described. Upper and/or lower guidance flows may also be provided as previously described.

The apparatus may further comprise means for producing said flow of hydrodynamically focused liquid and means for producing said flows of guidance liquid. Said means may comprise a respective pump for each flow. Said flow control means may then be constituted by means for controlling the rate at which each pump operates or by means for controlling the resistance to flow against which each pump works. The pumps may be positioned upstream or downstream of the flow channel(s).

Alternatively, the means for producing the required flow may be electrodes provided upstream and downstream of said intersection in the flow path for the hydrodynamically focused liquid and in the flow path of each guidance liquid and the means for controlling the flow may then be constituted by means for adjusting the respective voltages applied to the electrodes.

Such apparatus preferably further comprises a detector for detecting and/or quantitating at selected locations of said target surface products or results of the interactions of said hydrodynamically focused liquids.

The nature of the detector required will vary according to the nature of the interactions to be monitored but the detector may by way of example be a fluorescence detector, a radioactivity detector, a microscope, a confocal microscope, a luminescence detector, a spectrophotometer, or a photo-luminescence detector. An appropriately responsive photographic film, e.g. an X-ray film, may be used on the detector.

Means may be provided which is responsive to an output from such a detector provided as an input signal to said means and serving to deflect at least the guided flow to a selected outlet when an event is detected at said detector. Such an event may be the passage of a particle such as a cell which it is desired to capture or the passage of a bead bearing a marker which it is desired to capture.

The surface of the channel may be coated by a material having affinity for one or more materials which it is desired to remove from the guided stream. The guided stream may

contain particles such as cells which are substantially bigger than the materials to be removed.

The particles may be labelled with a detectable marker and different particles may be labelled with respective different markers, e.g. four fluorescent markers as used in the four colour DNA sequencing techniques, each having its own respective colour of fluorescence. Upon detection of a respective marker, the guided stream containing the marked particle may be deflected to a respective outlet. There may be as many outlets as there are types of marked particles to detect and there may be at least one additional outlet for flow during periods when no marked particle is detected.

Particles may be deflected to the appropriate outlet either by rapid rebalancing of the guiding flows or by a suction of liquid towards the desired outlet. This may be generated in a known manner by a piezo electric pump element in the outlet flow path.

In a further use of the methods taught herein, cells may be coated over the channel surface and may be stimulated by different materials flowed in respective lanes in one direction and tested for the result of said stimulation by different materials flowed in respective lanes in a crosswise direction. Alternatively, the cells may be stimulated by combinations of materials flowed in respective lanes in crossing directions.

Having regard to the small scale of the device, one may avoid using immortalised cells and use cells freshly taken in a biopsy.

Where the product of the method of the invention is an oligonucleotide array, electrodes may be provided below the

substrate surface or at the substrate surface in order to enable the user of the array to carry out electrically promoted hybridisation or denaturation, as known in the art.

According to a third aspect of the invention, there is provide a method for producing an interaction between a component of a liquid and a selected region of a target surface, comprising providing a said target surface in contact with a medium through which charged molecules can be caused to migrate, providing oppositely charged driving electrodes at opposed locations of said target surface in electrical contact with said medium to define a migration path between said driving electrodes, providing guiding electrodes of like charge on opposed sides of said migration path in electrical contact with said medium, supplying charged molecules to a starting location in said medium in said migration path and causing said molecules to migrate in said migration path away from one said driving electrode and towards the other said driving electrode whilst laterally electrodynamically focusing said migrating charged molecules to confine their movement within substantially less than the whole width of said migration path by the application of controlled voltages to said guiding electrodes so that the molecules migrate over said selected region of said target surface, and allowing said electrodynamically focused molecules to interact with said target surface. Such a method provides focusing by the use of electrophoretic force rather than by hydrodynamic focusing. The method may readily be adapted to provide electrodynamically focused migration first in one direction and subsequently in a second direction crosswise of the first direction in a similar manner to that

in which hydrodynamic focusing is used according to the second aspect of the invention. Interactions may be carried out and monitored generally as described above in the context of the first and second aspects of the invention.

The invention will be further described and illustrated by the following description of preferred embodiments with reference to the accompanying drawings, in which:

Figure 1 shows in plan view an example of apparatus in use in a method according to the first aspect of the invention;

Figure 2 shows in plan view apparatus according to the invention suited for use in accordance with the second aspect of the invention;

Figure 3 shows in plan view a detail of the apparatus of Figure 2 in use in a method in accordance with the second aspect of the invention;

Figure 4 shows schematically a section on the line IV-IV in Figure 2;

Figure 5 shows in plan view an alternative apparatus for use in accordance with the first aspect of the invention;

Figure 6 shows in plan view apparatus for use in accordance with the third aspect of the invention;

Figure 7 shows in schematic plan view at (a) and in cross-section at (b) a modified form of the apparatus of Figure 1 in which multiple guided streams are provided;

Figure 8 shows schematically in transverse cross-section at (a) in longitudinal cross-section at (b) and in plan view at (c) an alternative modification of the apparatus of Figure 1 in which a lower guiding flow is provided;

Figure 9 shows a fluid switching set-up for use with the apparatus of Figure 1;

Figure 10 shows the output of a digital camera monitoring an interaction in apparatus generally in accordance with Figure 1 (Example 1);

Figure 11 shows a similar interaction taking place in apparatus generally in accordance with Figure 2 (Example 2);

Figure 12 schematically illustrates apparatus used in cell sorting and purification; and

Figure 13 schematically illustrates apparatus used in Example 3.

As shown in Figure 1, apparatus for use in accordance with the method of the first aspect of the invention comprises a substrate 10. This is preferably chosen to be a material of high dimensional stability capable of being formed with a flow channel 12 of precisely defined and stable dimensions. The flow channel 12 takes the form of a trench having a floor 14 and side walls 16. A roof 18 (as in Figure 4) is provided over the trench by a plate suitably of the same material cemented over the trench.

The flow channel defines a flow path which extends left to right in the drawing and has at its upstream end three inlets for liquid. The centre inlet 20 is for a guided flow of hydrodynamically focused liquid 26. Each of the inlets 22 and 24 is for a respective guiding flow of guidance liquid 28. The end of the flow path provided with an outlet for the liquids at 30. Each of the inlets and the outlet has a respective flow control valve 32,34,36,38 (as in Figure 2).

The dimensions of the flow channel may be chosen within wide limits according to the nature of the interaction it is

desired to carry out. The main limiting factor will generally be the need to maintain substantially pure laminar flow so that the hydrodynamically focused liquid and the guidance liquids do not mix. Generally, provided the height of the flow channel is sufficiently small, say 40 μm, the width may be up to 10mm or more. Pillars may be provided extending between the floor and the roof of the flow channel to maintain the appropriate height spacing.

Generally, for a rectangular section channel, the appropriate length to height to width relationship is chosen to maintain laminar flow at the chosen flow rate, according to known formulae.

The methods described herein are operable over wide ranges of temperature, compatible with the liquids used remaining liquid.

Liquids are suitably driven through the apparatus by the use of syringe pumps or similar, suitably driven by stepper motors.

Care should generally be taken to avoid the formation of bubbles in all of the liquids used.

By way of example, there follows a description of the use of the apparatus so far described in making one dimensional and two dimensional oligonucleotide arrays, but the same principles may be readily adapted for making similar arrays of a different chemical nature, e.g. peptide arrays.

As the chemical techniques appropriate to synthesising oligonucleotides on a solid substrate are well known in the art no detailed account will be given here and the relevant principle will be explained with reference to a simplified account of the relevant chemical steps. Details of suitable

methods to be adapted for use according to the invention are for instance to be found in Carruthers M.H., Beaton, G., Wu J.V. and Wisher W., Methods in Enzymology (1992) 211:3-20. The surface of the floor of the flow channel is first made ready to react with a first nucleotide base in a known manner. A solution of a first desired base in a suitably reactive form (let us say adenine (A)) is introduced as flow 26 of hydrodynamically focused liquid and two flows of guidance buffer are introduced through inlets 22 and 24. The proportions of the total flow of guidance buffer introduced through these respective inlets are chosen so that the flow 26 is deflected laterally (vertically in the drawing) to the desired extent and in the desired direction from the centre line so that the hydrodynamically focused flow proceeds down a line within the flow channel and reaction of the base A with the surface is restricted to that line. The adenine solution may then be replaced with buffer so as to flush the adenine solution from the system and further reagents may be sent down the same line to carry out any deprotection or protection reactions required by the general reaction scheme at this stage.

Following this, the relative flows of guidance buffer may be readjusted whilst keeping the total flow rate of guidance buffer constant so as to redirect the flow 26 downwardly to the next desired level and a second nucleotide (let us say guanine (G)) may be reacted with the surface along a line at that level. The width of each line may be extremely small e.g. from 1 to 180 μm.

Similar parallel tracks of the bases cytosine (C) and thymine (T) may be laid down in similar fashion and the

sequence may be repeated as often as desired, thus providing a first layer of bound nucleotides in a one dimensional array.

Going back to the first track laid down (which was of base A) one can then lay down a track of a second base chosen from any one of A, T, C and G carrying out all necessary rinsing, protection and deprotection steps in accordance with the reaction scheme as one proceeds.

The process may be repeated as often as desired to produce a one dimensional oligonucleotide array in which each row contains a known oligonucleotide sequence.

By combining the processes described above with known photo-masking and photo-deprotection reactions a two dimensional array may be created.

Thus once the initial layer has been completed as previously described, a specific line of spots along the first track of base A laid down may be deprotected by a light activated photo-deprotection reaction. The next base introduced along that same line will then react only at the deprotected spots. A second base may then be reacted at a different set of spots deprotected along the same line through a different mask and so on such that along the first line laid down there are now numerous spots with each of the possible combinations AA, AT, AC, and AG.

This may then be repeated for each of the subsequent lines and over many layers to build a two dimensional array in which at each row/column intersection there is an oligonucleotide of a unique and known sequence.

The apparatus shown in Figures 2 to 4 lends itself to the building up of a two-dimensional array of oligo-

nucleotides or other polymers having a similar defined sequence of differing monomer subunits by a significantly different process in accordance with the second aspect of the invention.

The apparatus of Figure 2 resembles that shown in Figure 1 except in the following manner. A second flow channel 40 is defined like flow channel 12 by a trench in the substrate 10 having a floor 42 (Figs. 3 and 4) and side walls 44 (Fig. 3). The roof 18 is again provided and of course covers both flow channels.

The flow channel 40 defines a flow path which extends from the bottom to the top in the drawing and has at its upstream end three inlets for liquid. The centre inlet 48 is for a flow of hydrodynamically focused liquid 60. Each of the inlets 50 and 52 is for a respective flow of guidance liquid 62. The end of the flow path is provided with an outlet for the liquids at 54. Each of the inlets and the outlet has a respective flow control valve 66, 68, 70 and 72.

The flow channels 12 and 40 preferably, but not essentially, cross at right angles as shown and at their intersection, the floors of the flow channels share a region of the substrate 10 in common at 74 (Fig. 3) constituting a target surface. Preferably, the corners formed in the side walls of the flow channels at their intersection are radiused rather than hard.

Extending the previous account of building a one dimensional oligonucleotide array by way of example, after the first layer of lines of single nucleotide bases has been laid down as described with reference to Figure 1 (all conducted with inlets 48-52 and outlet 64 closed, one may

then shut the inlet valves 32-34 and outlet valve 38 and pass a flow of a hydrodynamically focused liquid 60 containing a selected nucleotide base through the flow channel 40 at a left-right position selected by the proportion of buffer liquid passed through each of the inlets 50,52. Subsequent lines of different bases may be laid down in selected positions such that at each row column intersection 76 (Fig. 3) one has a defined di-nucleotide sequence.

Further layers of rows may then be laid down alternately in the flow channels 12 and 40 to build oligonucleotides of any desired length with each row-column position containing a known oligonucleotide sequence.

By way of example, all possible 8-mers of oligonucleotides may be formed by putting down in a first layer 64 rows of A, followed by 64 rows of T, then 64 rows of C and 64 rows of G. Next one lays down 64 columns of each of A,T,C and G. Then one lays down four repeating sequences of 16 rows of each of A,T,C, and G and then four repeating sequences of 16 columns of A,T,C and G. This is followed by sixteen repeating sequences of 4 rows of each base and then by sixteen repeating sequences of 4 columns of each base. Lastly one lays down 256 single rows and then 256 single columns of each base in order. Each layer of rows or columns covers the entire area with 256 rows or columns in each layer.

If each row and column is made about 3 μm wide, one has patches of approximately 10 μm^2 arranged in an orthogonal 2-dimensional array, each containing a single unique 8-mer sequence at a known position within the array.

Where a number of adjacent rows of the same base are to be laid down, they need not be done individually. The hydrofocussed flow may be made broader to cover the desired area in one pass by adjusting the amounts of guide buffer and hydrodynamically focussed flow liquid.

If desired, more than one substrate 10 may be connected simultaneously to the control valves 32-38. There may be a stack of several substrates connected to said valves via suitable manifolds so that the operations described above can be carried out on all the substrates in the stack simultaneously. This is a particularly useful arrangement for producing numbers of identical substrates bearing lines or spots of molecules laid down on synthesised on the substrate.

As indicated in Figure 4, the apparatus may include a detector 80 for detecting and/or quantitating an interaction produced on the surface. For instance, the 8-mer array just described may be exposed to a labelled oligonucleotide of unknown sequence and the point in the array where hybridisation occurs may be determined using a suitable detector. The nature of the detector desired will vary widely according to the nature of the substances interacting on the surface and labelling methods used.

As shown in Figure 5, apparatus similar to that of Figure 1 may be driven by electro-osmotic force. Each inlet 20, 22, 24 and the outlet 30 is provided with a respective electrode 520, 522, 524 and 530. Suitably each electrode may be situated in contact with a reservoir from which the respective liquid is supplied or (in the case of the outlet 30) to which the liquid is directed. The surface of the

substrate bears an ionic charge as described above. This attracts a boundary layer of oppositely charged solvated ions in the liquid which are drawn by electrophoretic force toward the outlet electrode 530. Because the ions are solvated, this produces a flow of liquid. The flow rate from each inlet may be adjusted by control of the potential difference between its respective electrode and the outlet electrode 530. Typical operating voltages are from 1-10 kV. Due to the high resistance posed by the very small cross section of the flow channels, such high voltages may be used without producing excessive current and hence without excessive heat production.

The lateral position of the hydrodynamically focused flow is controlled by balancing the voltages applied to the guidance flow electrodes 522 and 524.

A similar modification may be applied to the apparatus of Figure 3.

If the surface of the substrate is such that electro-osmotic flow is not generated (or not to a significant extent), the apparatus shown in Figure 5 can nonetheless be operated according to the third aspect of the invention. Thus, if the surface of the substrate is not ionic, e.g. is coated with polyacrylamide, electro-osmotic flow will not occur and there will be no hydrodynamic focusing. However, charged molecules such as proteins or nucleic acids will migrate by electrophoretic forces from the inlet 20 towards the outlet 30 and will follow a focused laterally restricted track between the inlets 22 and 24 following the electric field vectors defined by the electrodes.

However, operating in this mode, there is no necessity to provide the guidance liquid on either side as shown in Figure 5. Instead, as shown in Figure 6, one may have a substrate 60 defining a target surface over which is a stationary liquid or gel medium. A pair of driving electrodes 62, 64 are positioned at opposite ends of the target surface, in electrical content with the medium. A migration path is thereby defined between the electrodes 62, 64. Although only one electrode 62 and one electrode 64 is shown, if desired a row of separate electrodes 62 or of electrodes 64 (or both) may be provided. On opposite lateral sides of the migration path between the electrodes 62, 64 are provided guidance electrodes 66, 68. Again, if desired a row of separate electrodes may be provided instead of the single elongate electrode shown.

Controlled electrical voltages are applied to the driving and guidance electrodes via a suitable controlled voltage source. As shown, the voltage applied to one of the driving electrodes is opposite in sign to that applied to the other three electrodes. Negatively charged molecules introduced at the driving electrode 62 will migrate in the medium towards the electrode 64 and will be laterally spatially confined or focused by the guidance electrodes 66, 68. The migration can be directed closer or further from the electrode 66 by suitable adjustment of the voltages applied to the two guidance electrodes 66, 68. The target surface defined between the electrodes may be similar in size to the target surface in the apparatus of Figures 1 to 4. Suitable voltages to drive the electrodynamically focused migration between the driving electrodes may be in the range of 0.5 to

10 kV/cm, e.g. 1-5 kV/cm. Whereas in the case of hydrodynamic focusing the channel dimensions are limited by the need to maintain laminar flow, in the case of purely electrophoretic driven electrodynamic focusing, the limiting factor may be the need to restrict heat generation.

The sign of the voltage applied to the electrodes in Figures 5 and 6 need not be as shown but may be chosen with regard to the nature of the substrate and the charge of the molecules to be focused.

After migration of molecules from electrode 62 to electrode 64 and interaction with the target surface, electrodes 66 and 68 may be used as driving electrodes and electrodes 62 and 64 may be used as guidance electrodes, with suitable reconfiguration of the applied voltages, so as to migrate molecules from electrode 66 to electrode 68, i.e. crosswise of the initially described migration.

Thus by way of further example, the surface may be coated uniformly with a first reactant, for instance an antibody, and a sample with a concentration of a binding partner for the antibody that varies over time may be progressively scanned over the surface using the apparatus of Figure 1 so that each row represents interaction between the flow and the surface over a defined time period. The concentration of the binding partner during each time period may then be determined by measuring the amount of binding in each row. As a variation of this, columns of different antibodies may be provided using the apparatus of Figure 2 and the sample may then be scanned down the surface as previously described so that each row now gives a measurement

of the amount of each respective antibody binding partner present in the sample during each time period.

In a further example of the use of the invention, the array of locations definable according to the practice of the invention may be used in a microscopic version of a conventional microtitre plate. Thus, virtual wells constituted by points in the array may be coated with an antibody or antigen (the whole surface of the substrate within the flow channel may be so coated) and the method described with respect to figure 1 may be used to expose respective rows of locations to a sample and to various concentrations of standard sequentially, followed by other conventional assay reagents with the resulting reaction product concentrations being read by the detector so that each row provides a number of 'repeats' corresponding to column locations within the row and the different rows provide calibration curve information as well as the result for the sample. Many variations on this theme will readily occur to those skilled in the art.

However, the methods of the invention may be used to address a sample to chosen areas of an array chip synthesised by other means, e.g. by spotting methods known in the art.

Generally the invention will have utility in clinical diagnostics, environmental monitoring, quality control, food technology and pharmaceutical screening, including use in toxicity studies, genomics proteomics cellular analysis. Sample materials that may be studied using apparatus and methods according to the invention will include body fluids, nucleotide based materials including DNA and RNA, proteins, peptides, polypeptides, cell culture products, waste water,

drinking water and ground water. In one aspect, cells are immobilised over the substrate surface and cells at different locations are treated with different reagents to see which produce a reaction in the cells, thus providing a mapping reflecting the phenotype of the cells. The methods and apparatus described will have wide application in combinatorial chemistry.

In Figure 7, the apparatus of Figure 1 is modified in that instead of a single inlet 20 for the guided stream, there are multiple inlets 20a, b,... for separate guided streams, each controlled by a respective inlet pump (not shown). Between each guided stream inlet 20a, 20b..., there is an inlet 23a,b... for a subsidiary guiding liquid stream. The guided streams 26a, b... are separated by flows 28a, b... of the guiding liquid and the guided streams and the subsidiary guiding streams all flow parallel to one another over a region of the floor of the channel dictated by the flow rate through the main guidance stream inlets 22, 24.

Each subsidiary guiding stream inlet may be provided with a respective controlling pump or they may be connect in common to a single pump.

In use, the desired flow rates may be established through the inlets 22 and 24 only to produce an interface between the guidance streams 28 at the desired lateral (up/down in the drawing) position in the channel 12. The flow through each of the inlets 20a, 20b... and each of the inlets 23a, 23b... may then be initiated, so that the streams of guided liquid flow at the previously established interface position, separated by the subsidiary guided streams. After the desired period of interaction between the guided streams

and the surface of floor 14, the flow through the inlets 20a, 20b... and each of the inlets 23a, 23b... may be stopped and the flow through the respective inlets 22 and 24 may be re-balanced to shift the interface between the flows 28 clear of the area previously worked on so that when flow through the inlets 20a, 20b... and each of the inlets 23a, 23b... is reinitiated, the guided streams interact with a fresh area of the floor 14. By this method of operation, the floor 14 may be 'painted' by any desired number of separate liquids much more quickly than in the apparatus of Figure 1. For instance, there may be three guided streams, each containing reactants for depositing a respective nucleotide A, T or G and layers of nucleotides may be reacted with the surface and then with growing nucleotide chains to rapidly build up a desired one dimensional array.

In Figure 8, the apparatus of Figure 1 is modified by the provision of a fourth inlet 23' for a guidance stream in front of (i.e. downstream from) an optional weir 25 so as to introduce a guidance stream 28' beneath the guided stream 26. A flow control pump (not shown) is provided for the inlet 23'.

By this means, the guided stream 26 can be pushed upwards for greater interaction with the upper surface of the channel or may be allowed to collapse downwards for contact and interaction with the floor 14 of the channel. Also, if particles of significant size such as cells or microbeads are being transported in the guided flow, the guiding flow 28' will provide additional dimensional flexibility in the flow 26, helping to prevent an agglomeration of particles clogging the flow path.

The fluid supply arrangements for use with the apparatus of Figure 1 are shown in more detail in Figure 9. These could be duplicated for use in the apparatus of Figure 2. A pump 35 for the guided flow stream is connected to feed through a sampling valve 92, which is a rotary valve that can be set to load a sample into a loop and to inject this into the guided flow steam with minimum interruption of the flow stream. Pumps 31 and 33 are provided for the guiding streams. A T junction 94 is provided immediately upstream of the inlet for the guided stream to the channel 12 so as to allow the guided stream flow to be diverted to waste rather than entering the channel 12. A three port, two way valve 96 is provided connected between the T junction, the outlet 30 and a drain line 98. In use, the valve 98 in the position illustrated allows sample to flow through the T junction for guided flow. When it is desired to cease passage of the sample, the pump 35 may be stopped or reversed. Switching valve 96 to connect the drain 98 to the T junction rather than to outlet 30 produces a situation in which the guiding flows are no longer able to exit via outlet 30 and therefore back flush through the T junction, there by clearing it of sample material. This prevents gradual leakage of sample material into the channel 12 during the period when the flow of sample is stopped.

The invention may be applied to depletion of unwanted DNA material from a sample stream containing biological cells and subsequent sorting of the cells into different containers in order to further analyse the DNA in the isolated cells.

In this instance, use is made of the apparatus shown in Figure 12.

A microchip structure may be formed as is described in Example 1 below, but formed with two outlets instead of a single outlet. The chip is connected up as shown in Figure 9, except that the extra outlet is connected to a piezo-element based valve 104. This valve keeps the outlet 100 closed, but has the capability to open when activated by a pulse. The piezo-element nature of the valve enables the valve to open and close very quickly in practice up to 1000 times per second.

The surface of the silicon chip may be altered by silanization using 3-aminopropyl-triethoxy-silane (4% (v/v) in dry acetone), thereby introducing positively charged amino groups on the entire chip surface.

In order to enable isolation of one group of biological cells from other cells in the sample preparation, the cells to be isolated from the other cells are labelled with a specific probe (e.g. an antibody) which is conjugated to a fluorochrome e.g. fluorescein.

The cell preparation including the fluorescein labelled cells are introduced into the sample stream and directed over the positively charged surface of the chip. By adjusting the flow rate of the guiding streams, the sample stream is narrowed down to around 5-25 μm, thereby allowing the biological cells to line up one after another in the sample stream.

As the sample stream is passing over the positively charged chip surface, molecules in the sample stream will, due to diffusion, interact with the surface. Due to their relatively large size, whole cells will not interact with the chip surface to any particular degree (the degree of

interaction can be controlled by adjusting the flow rate of the sample stream; a slow flow rate will give more interaction as the cell stays longer in the chip). The positively charged amino groups on the chip surface will "extract" negatively charged molecules such as DNA fragments from disrupted cells or other sources. Such depletion is very important in case a further analysis of the DNA in the isolated cells has to be performed. Generally in flow cytometry and cell sorting a variety of sample pre-preparation steps are needed before the cell sample can be introduced into the system. Especially in fields of applications where the sorted cell fraction is needed for PCR, sample impurities such as free nucleic acids (DNA, RNA) are problematic. The cell sample has to be washed before and/or after sorting by e.g. centrifugation steps, or DNA/RNA has to be removed by enzymatic treatment. In the presented embodiment the chip is used in combination with a fluidic cell sorter giving an on-chip cell sorting system having an integrated sample purification and washing step. In order to maximise the capacity for the depletion of DNA-fragments (and other negatively charged molecules) during the cell sorting process, the sample stream is guided over the entire chip surface by adjusting the speed of the guiding pumps. Thus, the chamber is used to capture undesired sample contents to the patterned surface within flow channel. Non-desired molecules such as DNA can be separated and withdrawn from the sample before sorting by binding them to the surface of the flow channel.

More generally by coating the micro channel with chemicals or electrodes specific chemical and mechanical

properties can be achieved. For instance, by coating of the micro channel with a cationic or anionic molecule a defined surface charge can be created, attracting certain molecules within the sample to bind or absorb to the surface.

Eletrophoretic forces created by an electrode (positioned within the flow channel) may be used to attract and attach corresponding molecules from the sample flow stream to the surface of the micro channel.

In this modification, the flow channel has electrodes positioned at the bottom of the flow channel. A voltage can be applied to the electrode giving a negative or positive bias. Various molecules, probes, receptors, indicators, etc. may be attracted to the attached at the surface of the electrode by applying a voltage to the electrode while a fluid with the corresponding probes, receptors, indicators, etc. flows in the flow channel. For example, DNA which has an overall negative charge is drawn to the electrode surface by a positive bias. To improve binding forces of the probe, e.g. DNA, electrodes coated with a specific layer or matrix, e.g. a polymer such as urethane or a reactive chemical group, can be used. Thus, an encapsulation or immobilisation of the molecule is achieved.

The cell or particle suspension is pumped, by e.g. syringe infusion pump, through the flow channel. The sample is observed and detected by a microscope 108 equipped with controlling means for controlling the two-way valve 104. These controlling means may comprise an optical detector, e.g. photomultiplier system (PMT). The objective is focused on the measuring point which is located inside the flow channel. The dimension of the measuring point is defined by

a pinhole positioned in front of the PMT and the magnification. The micro flow system is placed on a x-y-translational stage allowing one to move the micro flow system and to define an appropriate area as a measuring point. For light excitation several light sources can be used, e.g. the laser shown, or a tungsten lamp, or a photo diode. For optical detection a photomultiplier, CCD camera/chip or photo diode can for instance be used. For transmission of the light a fibre optic cable, a photo lens, an objective or a light microscope can be used. Various optical detection methods, e.g. fluorescence, absorbance, can be used.

Particles are physically separated (using hydrodynamic forces) according to the result of multiple simultaneous optical measurements on each particle. The photomultiplier (PMT) signals for each particle are transported to a pulse-height analyser also comprised within the controlling means. A selection circuit provides an activating signal whenever a specific particle exhibits photometric properties within a predetermined range. If the PMT signal for a specific particle exceeds a specific value an actuation pulse is produced. The trigger level and the duration for actuation is selected by the operator.

The sorting apparatus is designed to achieve a minimal dilution of the separated sample fraction. Hydrodynamic separation of particles can be performed due to the optical, electrical and other properties of the particle containing sample.

The particles suspended in a fluid medium are pumped through a separation flow chip and the cells are observed at

the optical axis of the photo detector, e.g. by a spectrophotometer or microscope, and flow to the separation junction. Unselected particles continue to flow out into the waste outlet 102. If a specific cell has optical properties causing an actuation signal, the valve opens causing the liquids to flow to the sort outlet 100 leading to capture inside the collecting channel attached to it. The actuation time or pulse by which the sort valve is switched on is made longer than necessary to ensure that the desired cell has been transported into the sorting channel.

The blocking valve 104, e.g. a piezoelectric drop-on-demand ink-jet printing valve, is attached to the sort outlet channel, allowing the blocking or opening of the stream passing through the sort outlet 100. The flow restriction in the waste outlet channel is much higher than the flow restriction of the sort outlet channel. This can be achieved by attaching a flow restrictor to the waste outlet channel 102. Thus if the sort outlet channel is not blocked the particle stream is deflected to the sort outlet channel. For analysis operation the sorting channel is continuously blocked by the sort valve 104. Thus, in this mode all the particles passing through the detection area which is defined by the slit in front of the photomultiplier are drained into the waste container. The trigger level and the duration for actuation is selected by the operator.

Thus for example, during the passage on the chip, each cell is addressed with laser light from laser 106 and the emitted light at 519 nm is captured in the microscope/photomultiplier 108. If no fluorescein molecules are present on the cells, the cells will flow out through outlet 102. If a

fluorescein molecule is detected, a pulse goes to the piezo-valve 104 causing it to open thereby directing the labelled cell out of the outlet 100. Hereinafter the cell can be collected and the DNA of the cell can be further analysed.

Very many other modifications and variations of this aspect of the invention as described above in its various exemplified embodiments will also readily occur to those skilled in the art. Instead of using a blocking valve, a syringe pump can be attached to the sort outlet channel allowing one to aspirate a defined volume from the stream into the sort outlet. As soon as a sorting event occurs the microsyringe pump is actuated for a short period of time (aspirating mode) and aspirates a defined volume of sample.

In a further example of an optical and mechanical arrangement of the sorter based on fluorescence detection illustrated schematically in Figure 12, the sample, e.g. particles in suspension, is guided and centred in a horizontal laminar sheath flow orthogonal to the optical axis. The particle stream is illuminated with the light of a mercury arc lamp passing excitation filters for e.g. fluorescein measurement. A dichroic mirror reflects the excitation light to the sorting chip via a e.g. 20x microscopy objective. The fluorescence light emission is collected by the same objective passing a dichroic mirror. Behind the mirror a slit works as field stop limiting the detection area to a small stripe. Each particle passing the detection field generates a short photomultiplier signal. The measuring signal is amplified and passed to a peak detector.

The system is designed so that the flow restriction in the waste outlet channel is bigger than in the sort outlet. Thus all liquids pumped through the separation flow channel are forced into the sort outlet. The sort outlet is connected to a two-way valve allowing one to control the direction of the sample flow. If a cell needs to be separated the valve is opened for a short period of time.

The sample enters the separation flow channel via the central inlet and is guided by two guiding buffers to the interrogation point and sorter junction. The sort outlet is normally blocked by the closed sort valve. Thus all flow stream is guided to the waste outlet. Unselected particles/cells continue to flow out into the waste outlet container. If a specific cell has optical properties causing an actuation signal, the sort valve opens for a short interval of time, causing the fluid to flow out through the sort channel into the collecting chamber. The delay time between detection and actuation of the sort valve is adjustable. The actuation time is adjusted long enough to ensure that a desired cell is guided to the sort outlet. The liquid volume guided to the sort outlet is defined by the time interval the valve is open and by the total flow rate. The actuation speed of the valve used in this device is 1500 MHz which corresponds to a minimal actuation time of 0.6 msec.

One further variation includes the use of several, e.g. 4 different fluorochromes (as known from so-called 4 colour sequencing of DNA molecules) for cell labelling and the sorting of cells into respective, e.g. 5 different outlets. Other variations include derivatization of the chip surface

to contain a molecular specific binding partner such as an antibody, a receptor or a DNA sequence. In such a variation, specific unwanted molecules can be depleted from the sample stream.

In a modification of the apparatus of Figure 12, the piezo valve at outlet 100 is substituted by an electrode positioned inside the flow channel close to the sorting outlet 100. Cells are separated by a transient electrical field which is switched on and pulsed for a short interval of time as soon as cell is needed to be sorted. The electrode creates a high frequency electrical field (e.g. 6 MHz). Living cells positioned in such a high frequency electrical field will be polarised and can be drawn towards the electrode by using an alternating field.

In a further modification of the apparatus of Figure 12, the bottom of the chamber is covered with an electrode. Molecules in the sample will be attracted (according to their net charge) to the surface of the channel by the electrode creating an electrical field.

Alternatively, the electrode can be positioned at one side of the channel. For instance, positively charged sample molecules may be drawn out of the focused sample flow stream by an electrical field and will be attached to the surface of the electrode. After analysis the surface can be purified by switching the voltage into a positive bias.

The invention will be further illustrated by the following Examples.

Example 1: Visualisation of fluorescein-labeled-biotin bound lane-wise on microchip surface via streptavidin.

A silica microchip having etched thereon a flow channel having three inlets and a single outlet, generally as shown in Figure 1 is coated with streptavidin. The chip is connected up as shown in Figure 9. The microchip provides a reaction chamber (1420 μm wide, 40 μm high and 5700 μm long) etched in the silicon and closed by bonding a cover lid of pyrex glass. Capillary connections from the syringe pumps to the reaction chamber was via 180 μm wide inlets. Surface derivatisation was performed by silanisation using 3-aminopropyl-triethoxy-silane (4% (v/v) in dry acetone) followed by incubation with glutardialdehyde (12.5% (v/v)) for 16 hours. A phosphate buffered saline (0.1M Na_2HPO_4, 0.15M NaCl, pH7.5) solution containing streptavidin (1 mg/mL) was reacted with the microchip surface for 15 minutes. Following a washing step, fluorescein-labeled-biotin was lane-wise guided over the chip surface by adjusting the speed of the individual pumps. The ratio of lane width and the width of the reaction chamber times the total flow rate gives the sample flow rate. Furthermore, by splitting the guiding liquid flow rate between both guiding streams we determined the position of each lane. The resulting lanes shown an average width of 65 μm. The picture in Figure 10 was acquired using a colour-CCD camera (CF15MC + control unit MCU, Kappa Messtechnik, Germany) mounted on a microscope (Eclipse E400 equipped with epi-fluorescence unit, Nikon).

Example 2: Crossed-field of immobilised lanes of fluorescein-labeled-biotin bound to a streptavidin coated microchip.

The microchip structure was formed as described in Example 1, but with inlet/outlets at three of the four corners of the pincushion square chamber with concave sides and an outlet at the remaining corner. The procedure was performed as described in Example 1. Following the generation of fluorescein lanes on the microchip surface in the X dimension using the sample- and guiding inlets X, another set of fluorescent lanes was immobilised in the Y dimension using the sample- and guiding inlets Y. The dimension of the reaction chamber was 2500 X 2500 μm (closest distance between opposite borders). The width of obtained immobilised lanes was around 50 μm near the centre of the chamber. The resulting pattern of fluorescein staining is seen in Figure 11.

Example 3: Interaction of a cell to a surface which is patterned with an array of reagents created by the method of the invention

A further use of the method and apparatus of the invention relates to the screening of anti-inflammatory drug candidates by cell rolling.

Cell rolling is the initial step in the inflammatory response. Adhesion molecules such as E-, P-, and L-Selectins which are secreted by endothelial cells and leukocytes upon activation are recognised by different membrane based receptors of leukocytes initiating cell rolling.

Cell rolling is caused by cycle of temporary adhesion and release of cells on a surface.

Adhesion inhibitors (acting as anti-inflammatory drugs) inhibit the rolling mechanism by blocking the adhesion molecules (Selectins). Anti-inflammatory drugs are interesting for treatment of arthritis, and different auto-immune diseases.

In an in vitro micro flow system, cell rolling is the interaction of a cell with a surface in a capillary system under continuous flow conditions. The flow velocity of an individual cell - which is dependent on the total flow rate, the viscosity, the diameter and roughness of the capillary - can be changed by the interaction of the cell with the specified surface. A temporary adhesion of a specific cell to the surface reduces the flow velocity of that specific cell within the flow system. The cell travels at a slower speed through the channel.

Figure 13 shows a schematic diagram of cell adhesion and inhibition in a selectin coated microflow chip of the type shown in Figure 1. The general procedure is as follows.

By the methods described above, the channel floor surface is patterned with different stripes of reagents as follows:-

Lane 1 & 5	E-Selectin
Lane 2 & 6	P-Selectin
Lane 3 & 7	L-Selectin
Lane 4 & 8	negative control

As an internal control a cell suspension is guided through the chamber for a defined period of time. The sample flow stream is hydrodynamically focused on stripe 1 to 4 in

turn. Cells and the Selectin interact and adhesion is observed within distinctive area of the chip.

The sample flow stream containing the cell suspension is substituted by a washing buffer. This washing buffer removes the remaining unbound cells out of the chamber. Cells interacting with the surface remain within the chamber as shown in the figure (cells bound to lane 3) and are flushed out with time.

For drug testing a cell suspension containing the drug/ reagent is guided through the chamber but hydrodynamically focused on stripes 5 to 8 in turn. No interaction of the cells with the selectin coated surface is observed in the presence of an effective drug.

The chamber is then washed as previously described.

In more detail, the flow chamber is coated with stripes of E-, P-, and L-Selectin, and a negative control using the hydrodynamic focusing procedure as described before.

Isolated neutrophils or lymphocytes are introduced into the chamber. The flow rate is chosen to get a shear rate which mimics blood flow in a vascular capillary (800 min^{-1}). While the cell sample is drawn at a continuous flow rate through the chamber the cells interact with the patterned surface.

For drug screening a mixture of the cell sample and the sample buffer containing the drug candidate are mixed and introduced into the chamber and guided via a second pattern, of E-, P-, and L-Selectin, and a negative control using the hydrodynamic focusing.

The rolling effect can be measured by an imaging system (e.g. light microscopic equipped with a CCD camera) which

detect the travelling time of an individual cell or counts the total amount of cells within a defined area (after removing the cell sample by a washing buffer) within the flow channel. Thus a response of a cell to a specific receptor can be determined. By using the system it is possible to determine a variety of different receptors (which are bound to defined areas of the channel surface) at a time.

Very many other modifications and variations of the invention as described above in its various exemplified embodiments will also readily occur to those skilled in the art.

CLAIMS

1. A method for producing an interaction between a hydrodynamically focused liquid or a component of said hydrodynamically focused liquid and a selected region of a target surface comprising:

providing said target surface as part of one of a plurality of surfaces together defining a flow path for liquid flow, the dimension of the surface containing the target surface transverse to the direction of said flow path serving to define the width of the flow path,

providing for said flow path a set of at least three fluid inlets and at least one fluid outlet such that a flow of said hydrodynamically focused liquid can be directed into the flow path through one said inlet guided between two flows of guidance liquid introduced via two other said inlets to leave said flow path through said at least one outlet,

providing for each pair of guidance liquid inlets flow control means such that the proportion of the total flow of guidance liquid introduced on each side of the said hydrodynamically focused liquid can be varied to position the flow of said hydrodynamically focused liquid laterally within the flow path and at a desired lateral position over the target surface, and

directing a flow of said hydrodynamically focused liquid and two flows of guidance liquid through respective ones of said inlets and along said flow path such that the flow of said liquid is directed over a selected region of said target surface having a width less than the width of the target surface and extending at a selected lateral position within said flow path controlled by selection of an appropriate flow ratio of guidance liquid introduced on either side of the flow of said hydrodynamically focused liquid,

and allowing said hydrodynamically focused liquid or a component thereof to interact with said selected region of said target surface.

2. A method as claimed in Claim 1, wherein said interaction between said hydrodynamically focused liquid and said target surface involves a chemical reaction.

3. A method as claimed in Claim 2, wherein said chemical reaction is an immunoaffinity reaction, a nucleotide hybridisation reaction, a chemical synthesis reaction, a chemical deprotection reaction, an enzyme catalysed reaction, an enzyme inhibition reaction.

4. A method as claimed in Claim 2, wherein said reaction comprises immobilising a first nucleotide or oligo-nucleotide on said surface.

5. A method as claimed in Claim 3, wherein said reaction comprises covalently adding a further nucleotide or oligonucleotide to a nucleotide or oligonucleotide already immobilised on said surface.

6. A method as claimed in Claim 2, wherein said reaction comprises immobilising a first amino acid residue or a peptide on said surface.

7. A method as claimed in Claim 3, wherein said reaction comprises covalently adding a further amino acid or peptide to one already immobilised on said surface.

8. A method as claimed in any preceding claim, wherein the flow of liquid through said flow path is at a Reynolds number of no more than 10.

9. A method as claimed in Claim 8, wherein the flow of liquid through said flow path is at a Reynolds number of no more than 5.

10. A method as claimed in Claim 8, wherein the flow of liquid through said flow path is at a Reynolds number of no more than 1.

11. A method as claimed in any preceding claim, wherein said flow channel is produced by etching of a glass, fused silica or silicon substrate.

12. A method as claimed in any one of Claims 1 to 10, wherein said flow channel is produced by injection moulding of plastics to form a shaped substrate.

13. A method as claimed in any preceding claim, wherein following the production of said interaction at said selected region of said target surface, a second interaction is conducted between a product of said first interaction and a second hydrodynamically focused liquid or component thereof at a selected sub-region forming part of said selected region by a method comprising:

providing a second plurality of surfaces together defining a second flow path for liquid flow such that one of said surfaces intersects and has a portion in common with said target surface, the dimension of said one surface transverse to the direction of said second flow path defining the width of the second flow path,

providing for said second flow path a second set of three fluid inlets and at least one fluid outlet such that a flow of said second hydrodynamically focused liquid can be directed into the flow path through a said inlet guided between two flows of guidance liquid introduced via two other said inlets to leave said flow path through said at least one outlet,

providing for each pair of guidance liquid inlets of the second flow path flow control means such that the proportion of the total flow of guidance liquid

introduced on each side of the said second hydrodynamically focused liquid can be varied to position the flow of said second hydrodynamically focused liquid laterally within the second flow path, and

directing a flow of said second hydrodynamically focused liquid and two flows of guidance liquid through respective ones of said inlets and along said second flow path such that the flow of said liquid is directed over a selected sub-region of said common portion of said target surface having a width less than the width of the second flow path and lying at a selected lateral position within said second flow path controlled by selection of an appropriate flow ratio of guidance liquid introduced on either side of the flow of said second hydrodynamically focused liquid,

and allowing said second hydrodynamically focused liquid or a component thereof to interact with the product of said first interaction on said selected sub-region of said target surface.

14. A method for producing an interaction between hydrodynamically focused liquids or components of said hydrodynamically focused liquids at a selected region of a target surface comprising:

providing said target surface at an intersection formed by two crossing flow paths defined by respective sets of flow path bounding surfaces,

each said set of bounding surfaces comprising a surface having a width that defines the width of its respective flow path, the target surface being defined by the intersection of said width defining surfaces,

providing for each flow path a set of three fluid inlets and at least one fluid outlet such that for each flow path, a flow of hydrodynamically focused liquid can be directed into the flow path through one said inlet guided between two flows of guidance liquid introduced via two other said inlets to leave said flow path via said at least one outlet,

providing for each pair of guidance liquid inlets flow control means such that the proportion of the total flow of guidance liquid introduced on each side of the hydrodynamically focused liquid can be varied to position the flow of hydrodynamically focused liquid laterally within the respective flow path,

directing a flow of a first hydrodynamically focused liquid along one of said intersecting flow paths to carry out a first interaction between said first hydrodynamically focused liquid or a component thereof and the target surface along a line extending at a selected lateral position within said flow path

controlled by selection of an appropriate flow ratic of guidance liquid introduced on either side of the flow of hydrodynamically focused liquid, said interaction producing a product on said target surface,

stopping flow through said one flow path,

directing a flow of a second hydrodynamically focused liquid along the other one of said intersecting flow paths to carry out a second interaction between said second hydrodynamically focused liquid or a component thereof and the product of said first interaction on said target surface at a point within the intersection of the flow paths lying along a line extending at a selected lateral position within said other flow path controlled by selection of an appropriate flow ratio of guidance liquid introduced on either side of the flow of said second hydrodynamically focused liquid,

whereby said second interaction takes place at a selected location within the area of intersection of the two flow paths defined by the selected lateral positions of said hydrodynamically focused liquid flows.

15. A method as claimed in any preceding claim, wherein said flow of hydrodynamically focused liquid and said flows of guidance liquid are electro-osmotic flows.

16. Apparatus for use in producing an interaction between hydrodynamically focused liquids or components of said

hydrodynamically focused liquids at a selected region of a target surface comprising:

a substrate defining said target surface at an intersection formed by two crossing flow paths defined by respective sets of flow path bounding surfaces of the substrate,

each said set of bounding surfaces comprising a surface having a width that defines the width of its respective flow path, the target surface being defined by the intersection of said width defining surfaces,

a set of at least three fluid inlets and at least one fluid outlet associated with each said flow path such that for each flow path, a flow of hydrodynamically focused liquid can be directed into the flow path through one said inlet guided between two flows of guidance liquid introduced via two other said inlets to leave said flow path via said at least one outlet, and

flow control means associated with each pair of guidance liquid inlets such that the proportion of the total flow of guidance liquid introduced on each side of the respective hydrodynamically focused liquid can be varied to position the flow of hydrodynamically focused liquid laterally within the respective flow path.

17. Apparatus as claimed in Claim 16, further comprising a detector for detecting and/or quantitating at selected locations of said target surface products of the interactions of said hydrodynamically focused liquids.

18. A method for producing an interaction between a component of a liquid and a selected region of a target surface, comprising providing a said target surface in contact with a medium through which charged molecules can be caused to migrate, providing oppositely charged driving electrodes at opposed locations of said target surface in electrical contact with said medium to define a migration path between said driving electrodes, providing guiding electrodes of like charge on opposed sides of said migration path in electrical contact with said medium, supplying charged molecules to a starting location in said medium in said migration path and causing said molecules to migrate in said migration path away from one said driving electrode and towards the other said driving electrode whilst laterally electrodynamically focusing said migrating charged molecules to confine their movement within substantially less than the whole width of said migration path by the application of controlled voltages to said guiding electrodes so that the molecules migrate over said selected region of said target surface, and allowing said electrodynamically focused molecules to interact with said target surface.

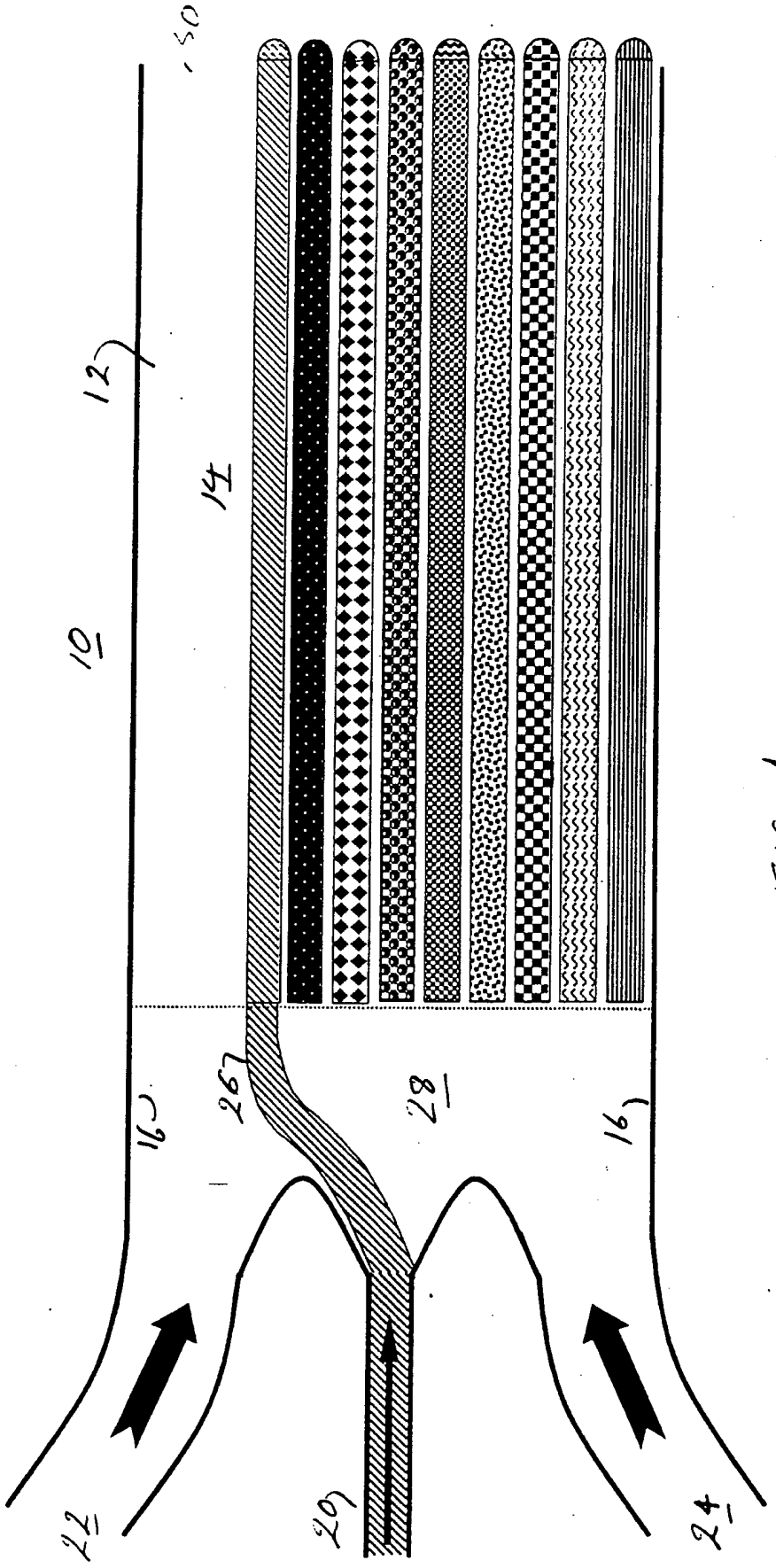


FIG 1

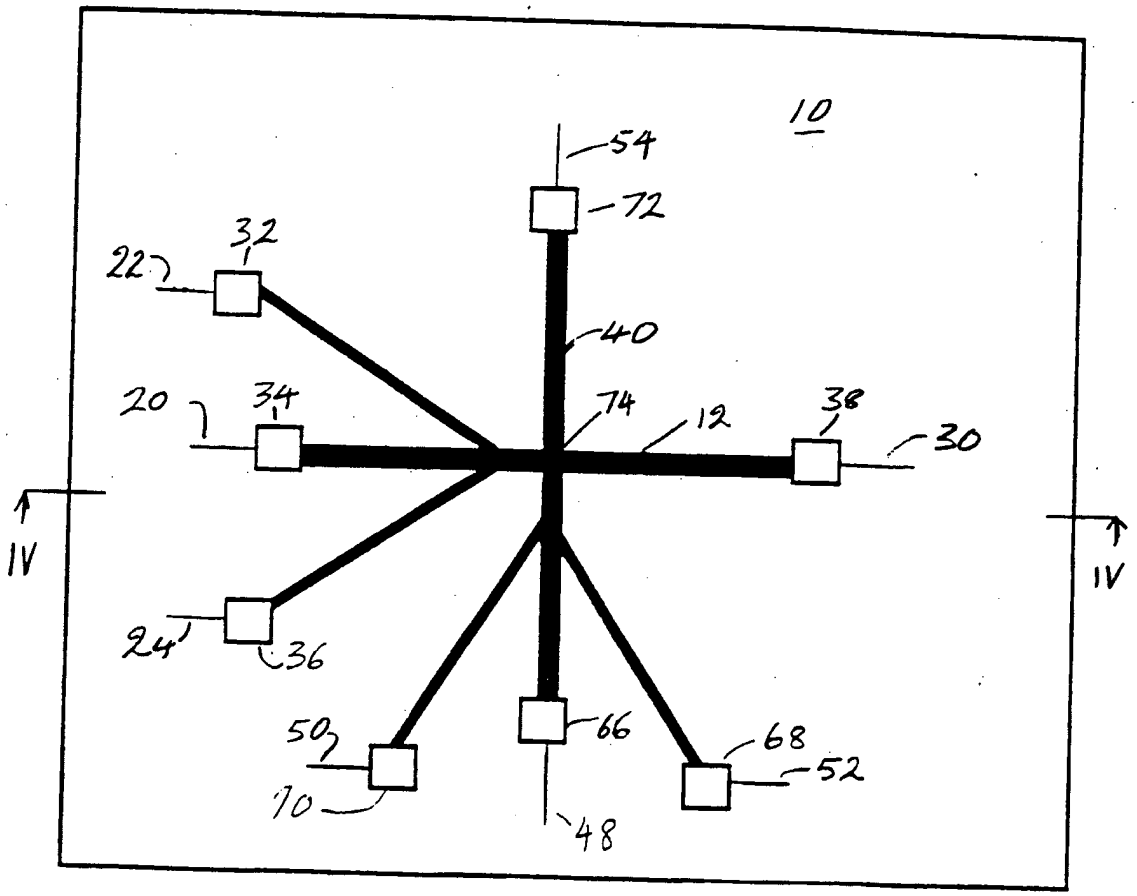


Fig 2

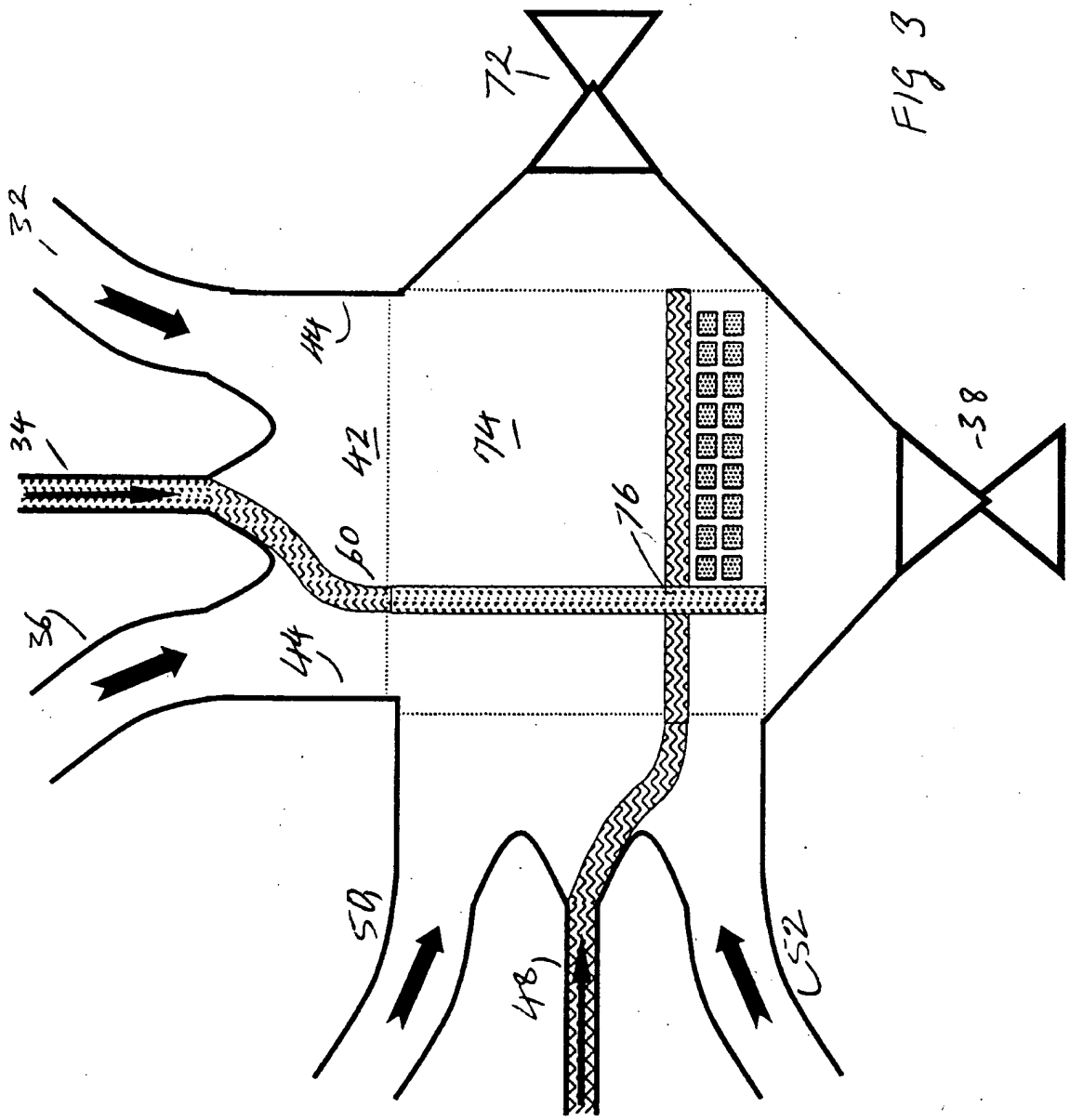


FIG 3

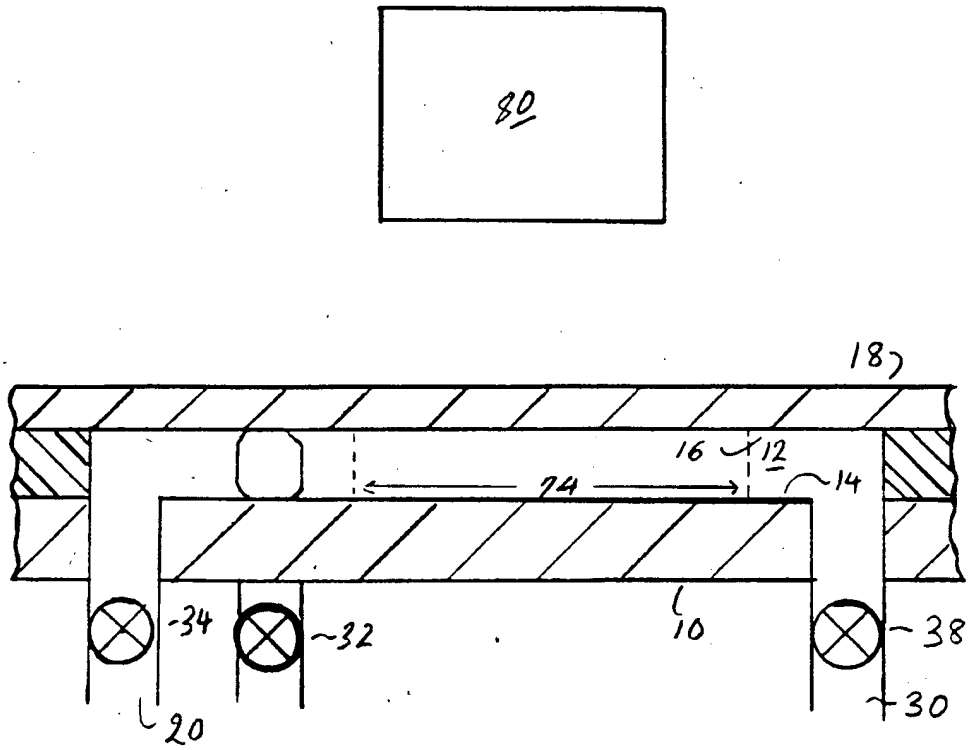


FIG 4

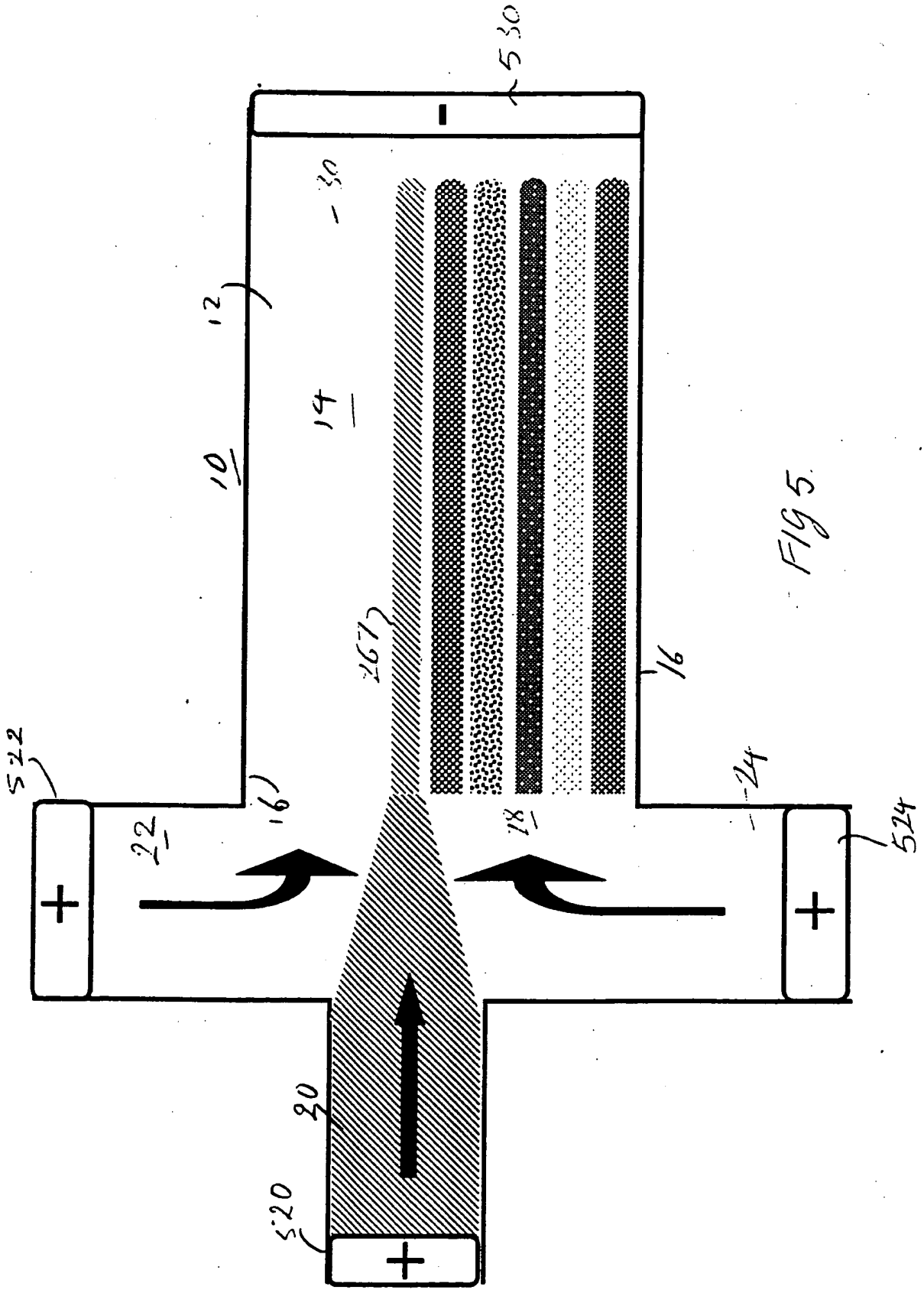


FIG 5

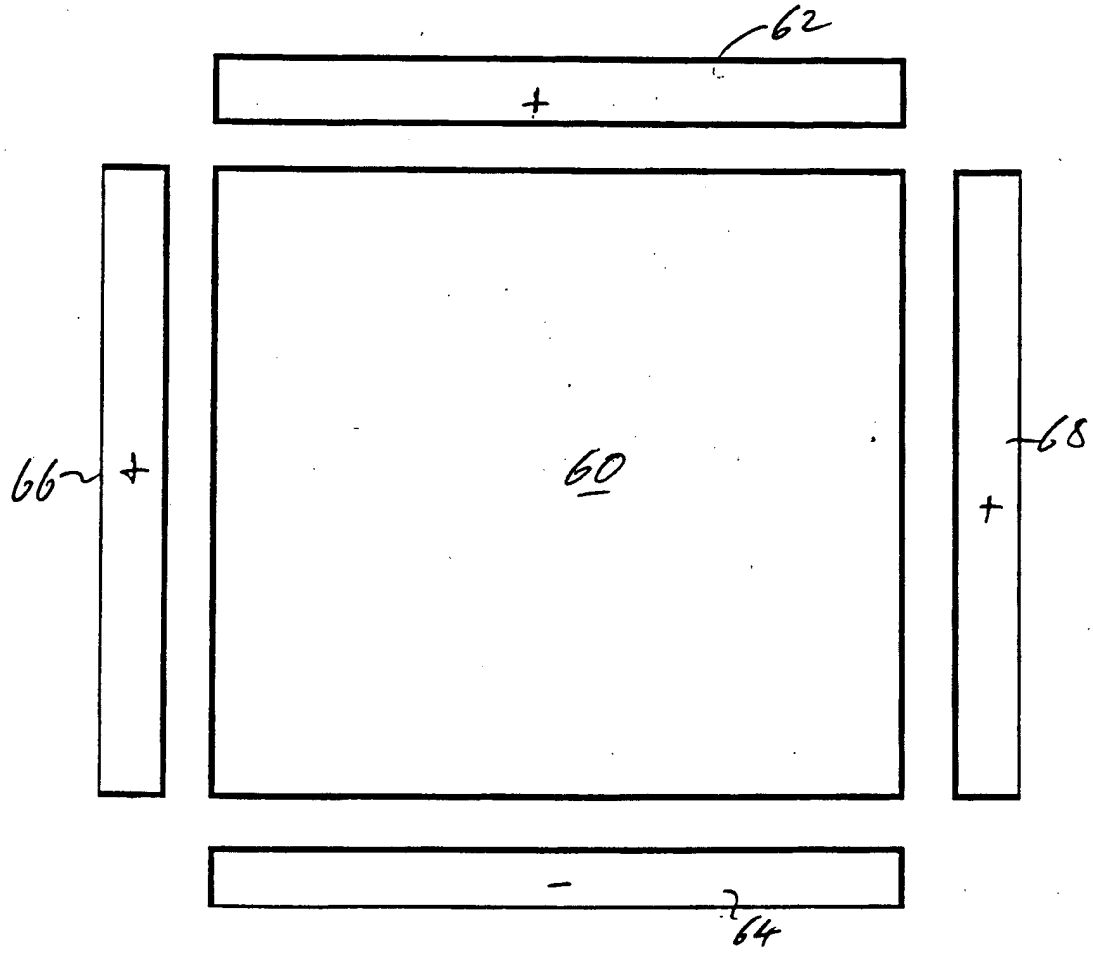


Fig 6

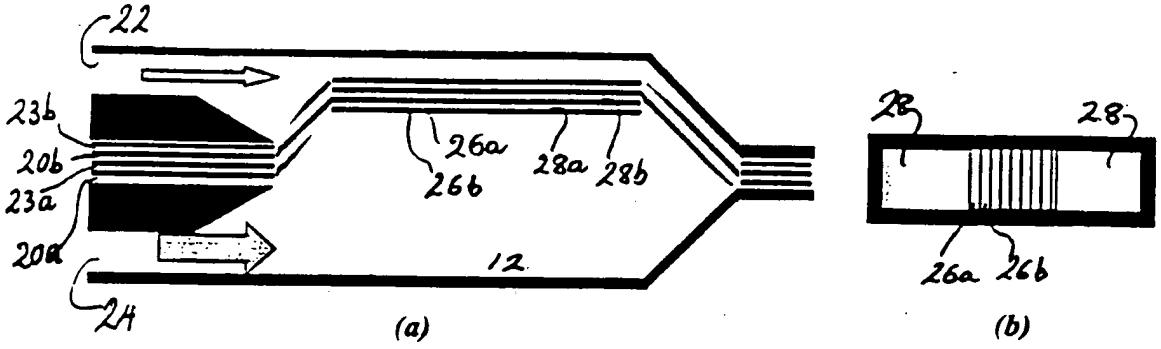
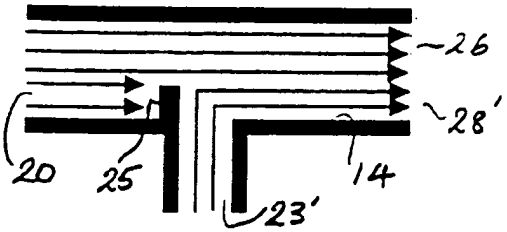
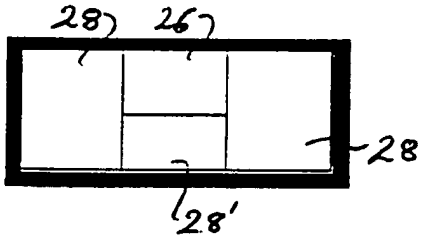

22
23b
20b
23a
20a
24
26a
26b
28a
28b
12
(a)
28
28
26a
26b
(b)

Fig. 7


26
28'
20
25
14
23'
(a)


28
26
28
28'
(b)

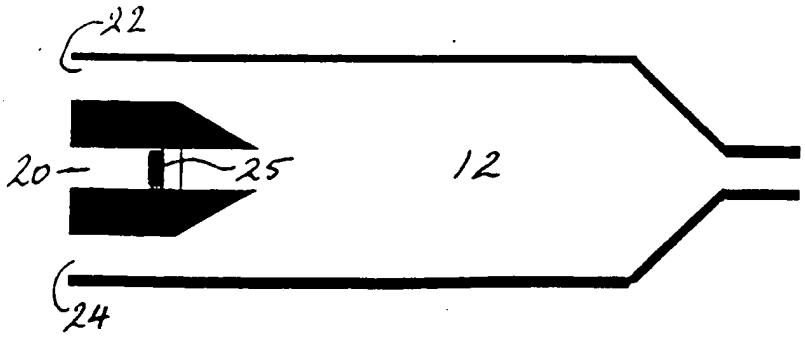

22
20
25
12
24
(c)

Fig. 8

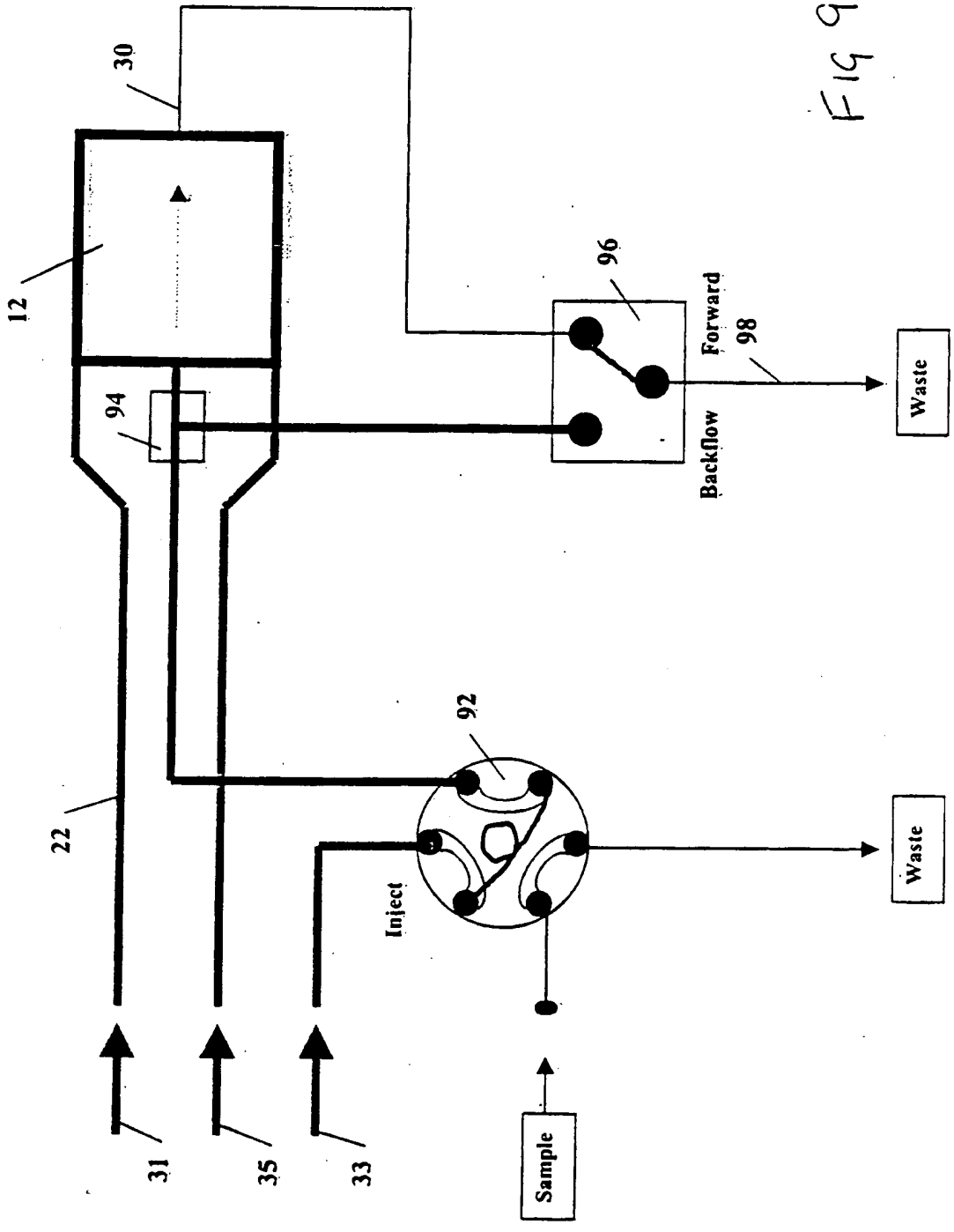

30
12
96
Forward
98
Waste
94
Backflow
92
22
Waste
Inject
31
35
33
Sample

Fig 9.

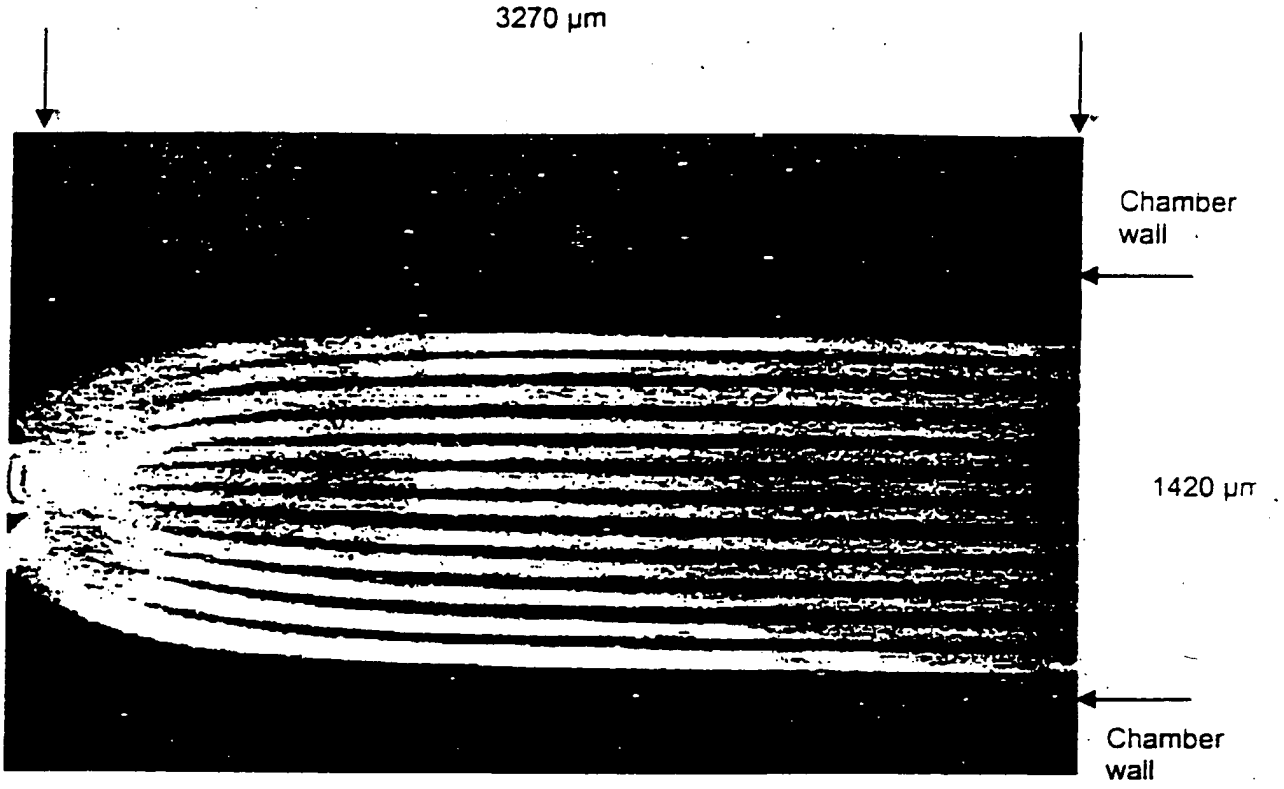


Figure 10

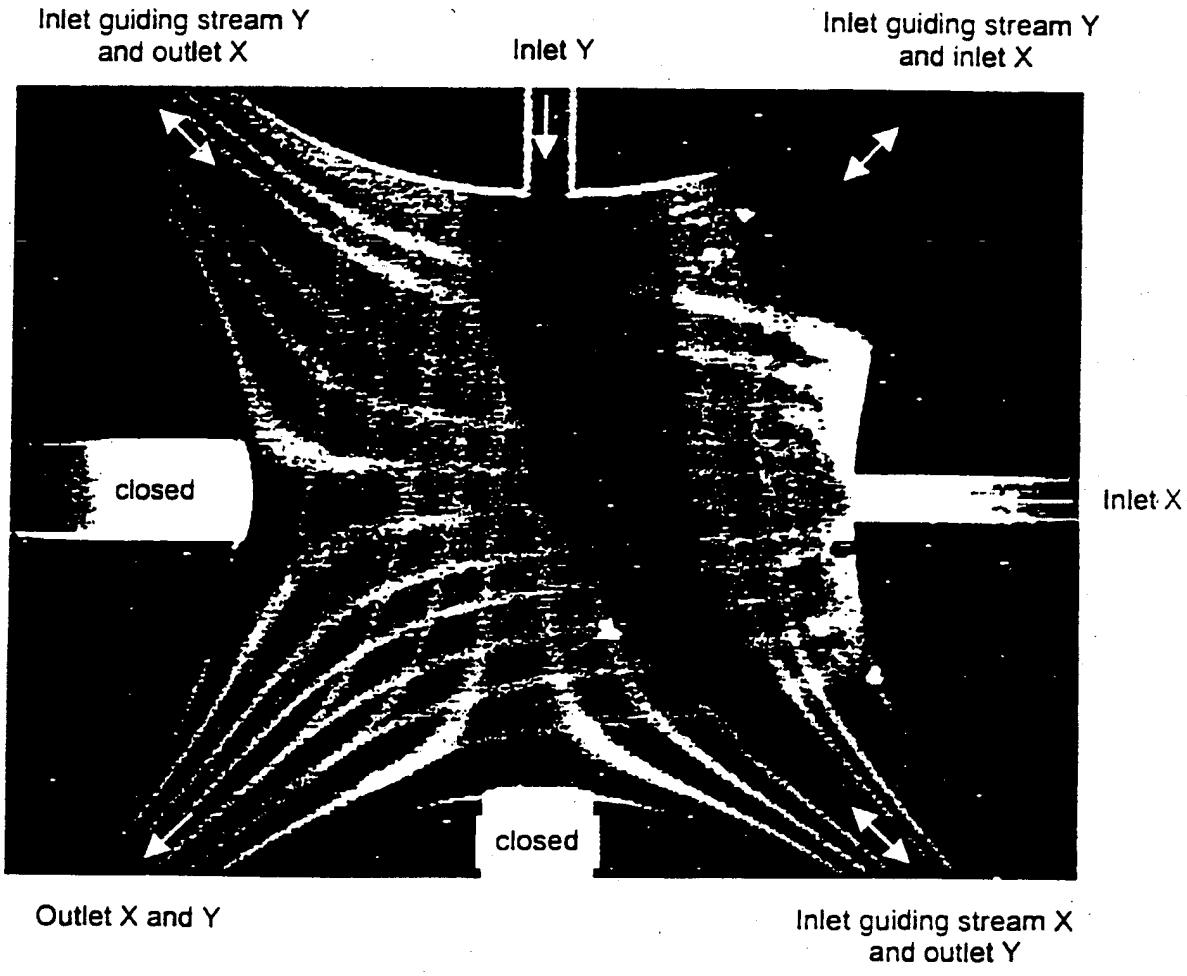

Inlet guiding stream Y and outlet X
Inlet Y
Inlet guiding stream Y and inlet X
closed
Inlet X
closed
Outlet X and Y
Inlet guiding stream X and outlet Y

Figure 11

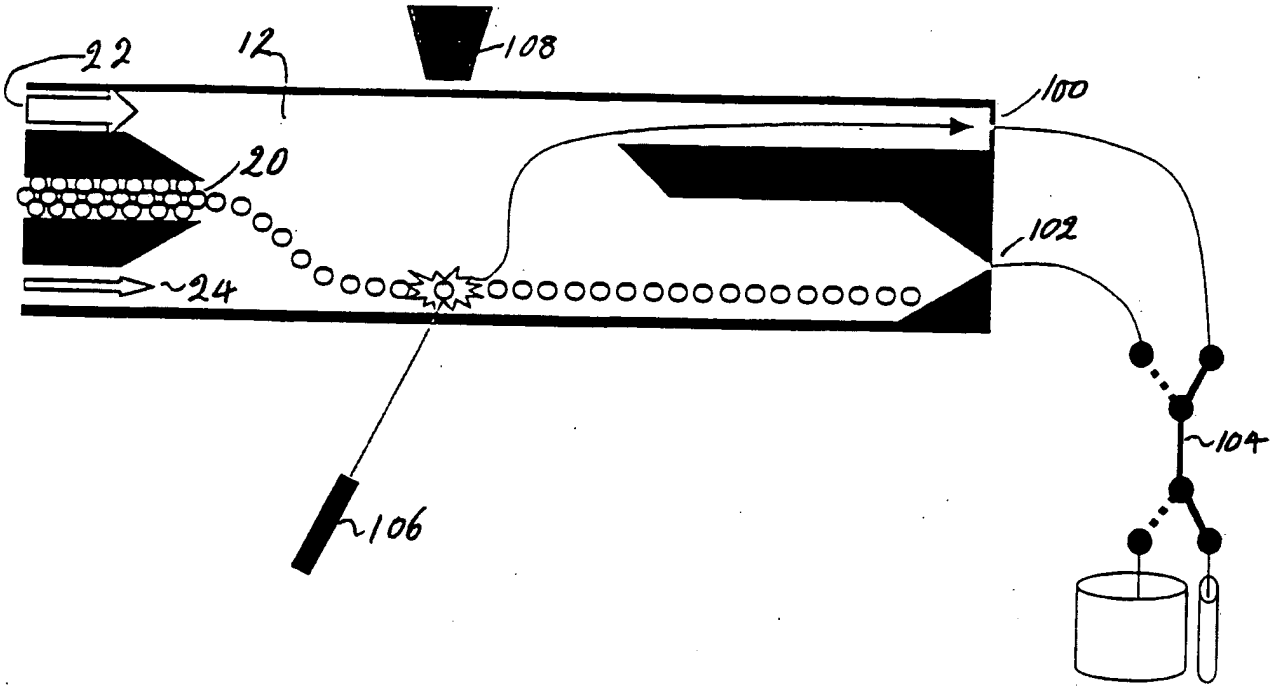


FIG 12

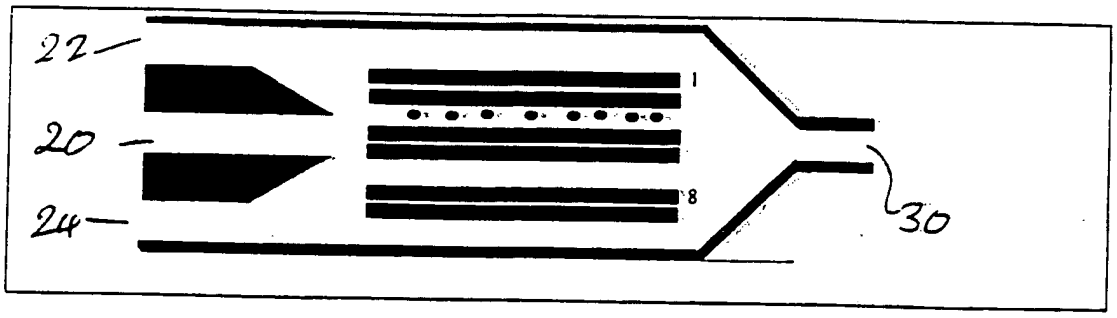


FIG 13